TRADE SECRET

Study Title

H-28072: Local Lymph Node Assay (LLNA) in Mice

TEST GUIDELINES: U.S. EPA Health Effects Test Guidelines
OPPTS 870.2600 (2003)

OECD Guideline for the Testing of Chemicals
Section 4 (Part 429) (2001)

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ORIGINAL REPORT COMPLETED: July 2, 2007

REPORT REVISION 1: October 1, 2007

PERFORMING LABORATORY: E.I. du Pont de Nemours and Company
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Newark, Delaware 19714
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LABORATORY PROJECT ID: DuPont-22616

WORK REQUEST NUMBER: 17199

SERVICE CODE NUMBER: 1234

SPONSOR: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards, which are compatible with current OECD Good Laboratory Practices, except for the item documented below. The item listed does not impact the validity of the study.

The test substance and control preparations used in the study were not analyzed for concentration, uniformity, or stability. The procedures used by trained staff to prepare the dosing preparations ensured:

- the accuracy of concentration because all preparations were performed using calibrated pipettes,
- uniformity and stability because each preparation was formulated daily just prior to dosing, and
- each vehicle and positive control group gave expected results in the study.

Study Director: Denise Hoban 01 Oct 2007

Denise Hoban, B.A., MLT (ASCP) — Date
Staff Medical Technologist and Supervisor

QUALITY ASSURANCE STATEMENT

Work Request Number: 17199
Service Code Number: 1234

Key inspections for DuPont work request 17199, service code 1234 were performed for the tasks completed at DuPont by the Quality Assurance Unit of DuPont and the findings were submitted on the following dates.

Phase Audited	***Audit Dates***	***Date Reported to Study Director***	***Date Reported to Management***
Protocol:	March 26, 2006	March 26, 2006	March 26, 2006
Conduct:	April 02, 2007	April 02, 2007	April 02, 2007
Report/Records: (Final Report Revision #1)	April 24, 2007 September 21, 2007	April 24, 2007 September 21, 2007	April 25, 2007 September 21, 2007

Reported by: Kenneth Granville — 27 Sept 2007

Kenneth Granville
Quality Assurance Auditor

Date

CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

Reveiwed by: Carol Carpenter — 01 Oct 2007
Carol Carpenter, B.A.
Senior Staff Toxicologist
Date

Issued by Study Director: Denise Hoban — 01 Oct 2007
Denise Hoban, B.A., MLT (ASCP)
Staff Medical Technologist and Supervisor
Date

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STUDY INFORMATION

Substance Tested:
- HFPO Dimer Acid Ammonium Salt
- 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propionic acid, ammonium salt
- 62037-80-3 (CAS Number)
- H-28072

Haskell Number: 28072

Composition:

82.6%	Ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propionate*
13.9%	Water
3.5%	Ammonium
0.41%	Organic Impurities

* Note: The Ammonium-2,3,3,3-tetrafuoro-2-(heptafluoropropoxy) propionate component (HFPO Dimer ammonium salt) contains 0.1 ppm HFPO trimer ammonium salt.

Purity: See composition, above

Physical Characteristics: Clear and colorless concentrated aqueous solution

Stability: The test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.

Study Initiated/Completed: March 21, 2007 / (see report cover page)

Experimental Start/Termination: March 28, 2007 / April 3, 2007

REASON FOR REVISION 1

The report was revised as follows:

Page	
7	Study Information Page - revised to reflect updated Certificate of Analysis
8	Summary - added information about animal fates.
14	Local Lymph Node Assay - clarified procedures.
16	Results and Discussion - added information about animal fates.
22	Table 4 - added information about animal fates.
24	Certificate of Analysis - updated with revised analysis.

The following pages were revised to reflect these changes.

Page	
1	Title Page
5	Table of Contents

SUMMARY

The objective of this study was to evaluate the potential of H-28072 to produce a dermal sensitization response in mice using the local lymph node assay (LLNA). Five groups of 5 female CBA/JHsd mice were dosed for 3 consecutive days with 0% (vehicle control), 5%, 25%, 50%, or 100% H-28072 on both ears. N,N-dimethylformamide (DMF) was used as the diluting vehicle. One group of 5 female mice was dosed for 3 consecutive days with 25% hexylcinnamaldehyde (HCA) in DMF as a positive control. On test day 5 of the assay, mice received ^{3}H-Thymidine by tail vein injection and were sacrificed approximately 5 hours later. The cell proliferation in the draining auricular lymph nodes of the ears from the test substance groups was then evaluated and compared to the vehicle control group.

No statistically significant differences in mean body weights compared to the vehicle control group were observed at any test concentration. A statistically significant increase in mean body weight gains compared to the vehicle control group was observed at the 25% test concentration. No clinical signs of toxicity were observed in the study. One mouse in the 100% test concentration group was found dead on test day 3. Gross examination showed bright red lungs.

Although statistically significant increases in cell proliferation measurements compared to the vehicle control group were observed at the 50% and 100% test concentrations, stimulation

indices (SIs) of less than 3.0 were observed at all test concentrations of H-28072. Therefore, the EC3 value (the estimated concentration required to induce a threshold positive response, i.e., SI = 3) for the test substance under the conditions of this study was not calculable. A 25% concentration of the positive control, HCA, produced a dermal sensitization response in mice. Therefore, the LLNA test system was valid for this study with H-28072. Under the conditions of this study, H-28072 did not produce a dermal sensitization response in mice.

Based on these data, H-28072 is not a dermal sensitizer.

INTRODUCTION

The purpose of this study was to examine the dermal sensitization potential of H-28072 using the mouse local lymph node assay (LLNA).[1,2,3,4,5] Following the topical application of the test substance to the dorsal side of both ears, the dermal sensitization potential of the test substance was evaluated by measuring the proliferation of lymphocytes (via radiolabel uptake) obtained from the auricular lymph nodes (i.e., the lymph nodes that drain the ears). Results were compared to the vehicle control group.

Because H-28072 is a liquid and did not appear to have severe skin-irritating capability (pH 10), the 100% concentration was chosen as the high dose. For subsequent concentrations, the test substance was prepared in N,N-dimethylformamide (DMF).

STUDY DESIGN

The study design was as follows:

Group	Number/ Group	Dosage (%)[a]
1	5	0 (Vehicle Control)
2	5	5
3	5	25
4	5	50
5	5	100
6	5	25 (Positive Control)

a % = percent of test substance in vehicle control (e.g., 100% = 1 g/mL, or neat test substance)

Study Parameter	Frequency
Body Weight	Test days 0 and 5
Daily Animal Health Observations	At least once daily
Careful Clinical Observations	Prior to dosing and prior to sacrifice
Dosing	Test days 0-2
Days of Rest	Test days 3-4
Injection of Radioactivity	Test day 5
Removal of Lymph Nodes	At sacrifice (test day 5)
Disintegrations per minute (dpm) data	Test day 6

MATERIALS AND METHODS

A. Test Guidelines

The study design complied with the following test guidelines:

- U.S. EPA, OPPTS 870.2600: Skin Sensitization, *Health Effects Test Guidelines* (2003)

- OECD, Section 4 (Part 429): Skin Sensitisation: Local Lymph Node Assay, *Guideline for the Testing of Chemicals* (2001)

B. Vehicle Control

The vehicle control, DMF, was purchased commercially and used for all test substance dilutions on all dose days. Impurities in the vehicle control were not expected to interfere with the study results. The vehicle control was assumed to be stable under the conditions of the study. No evidence of instability, such as a change in color or physical state, was observed.

C. Test Substance

(Appendix A)

The test substance, H-28072, was supplied by the sponsor as a clear and colorless concentrated aqueous solution. The sample was stored according to the sponsor's instructions. The test substance appeared to be stable under the conditions of the study. No evidence of instability, such as a change in color or physical state, was observed.

The test substance was prepared as a solution in the vehicle control according to the concentrations listed in the Study Design, except for the 100% concentration, which was used neat.

D. Positive Control

The positive control, hexylcinnamaldehyde (HCA), was purchased commercially. Any available information on the positive control was included in the study records. Impurities in the positive control were not expected to interfere with the study results. The positive control appeared to be stable under the conditions of the study. No evidence of instability, such as a change in color or physical state, was observed.

A 25% HCA solution in the vehicle control was blended using a vortex mixer and stored in a vial protected from light until dosing was completed.

E. Dosing Preparations and Analyses

Prior to study start, a quantity of the test substance was evaluated for solubility in a particular vehicle. The control and test substance concentrations and method of preparation were based on solubility information. All dose preparations were formulated fresh daily.

Dose preparations were not analyzed for homogeneity or accuracy of concentration. The dose preparation procedures were believed to provide homogeneous mixtures at the targeted concentrations. In the absence of visible change in color or physical state, all dose preparations were assumed to be stable throughout the study.

All dose preparations applied to the test site were assumed to be available for absorption by the test system unless otherwise indicated in the study records. All calculations and the evaluation of effects were based on the applied dose.

F. Test System

Female (nulliparous and non-pregnant) CBA/JHsd mice were received from Harlan Sprague Dawley, Frederick, Maryland, U.S.A.

The CBA/JHsd mouse was selected to conduct the LLNA because it is the strain recommended in the test guidelines. In addition, Haskell Laboratory has extensive LLNA experience with the CBA/JHsd mouse strain, and this strain has undergone extensive interlaboratory validation with the LLNA.[1,2,3,4,5]

G. Animal Husbandry

1. Housing

All animals were housed in stainless steel, wire-mesh cages suspended above cage boards. During quarantine, animals were housed in pairs. After assignment to groups, and during the dosing and resting phases of the study, animals were housed singly. After final weighing (test day 5) until sacrifice, animals were housed one group per plastic shoebox cage with appropriate bedding.

2. Environmental Conditions

Animal rooms were maintained at a temperature of 18-26ºC and a relative humidity of 30-70%. Animal rooms were artificially illuminated (fluorescent light) on an approximate 12-hour light/dark cycle. Any excursions outside of these ranges were of insufficient magnitude and/or duration to have adversely affected the validity of the study.

3. Feed and Water

All mice were provided tap water *ad libitum*. All mice were fed PMI® Nutrition International, LLC Certified Rodent LabDiet® 5002 *ad libitum*.

4. Animal Health and Environmental Monitoring Program

As specified in the Haskell Laboratory animal health and environmental monitoring program, the following procedures are performed periodically to ensure that contaminant levels are below those that would be expected to impact the scientific integrity of the study:

- Water samples are analyzed for total bacterial counts, and the presence of coliforms, lead, and other contaminants.
- Samples from freshly washed cages and cage racks are analyzed to ensure adequate sanitation by the cagewashers.

Certified animal feed is used, guaranteed by the manufacturer to meet specified nutritional requirements and not to exceed stated maximum concentrations of key contaminants, including specified heavy metals, aflatoxin, chlorinated hydrocarbons, and organophosphates. The presence of these contaminants below the maximum concentration stated by the manufacturer would not be expected to impact the integrity of the study.

The animal health and environmental monitoring program is administered by the attending laboratory animal veterinarian. Evaluation of these data did not indicate any conditions that affected the validity of the study.

H. Pretest Period

Upon arrival at Haskell Laboratory, all mice were:

- quarantined for a minimum of 6 days.
- identified temporarily by the presence or absence of a colored tail mark and cage identification.
- weighed 3 times during quarantine and twice prior to initiation of dosing.
- observed with respect to weight gain and any gross signs of disease or injury.

The mice were released from quarantine on the basis of body weights and clinical signs of all mice.

I. Assignment to Groups

Mice, selected based upon adequate body weight gain and freedom from any ear abnormalities (e.g., torn, scratched) or clinical signs of disease or injury, were distributed into study groups as designated in the Study Design. Prior to study start, each mouse was assigned to a group using a randomly generated, computer-based algorithm such that individual pretest body weights did not vary more than 20% of the group mean.

At grouping, each mouse was assigned an animal number. The animal number was marked on the tail of each mouse with solvent-resistant ink. Color-coded labels were attached to the animal rack above each cage prior to dosing and included the group number, the animal number, the dose concentration, and the dose substance.

At study start (test day 0), mice were approximately 9 weeks old and weighed between 19.9 and 23.8 grams.

Mice not assigned to a test group were released for other laboratory purposes or sacrificed by carbon dioxide asphyxiation and discarded without anatomic pathology evaluation, at the discretion of the study director.

J. Body Weights

All mice were weighed on test day 0 and prior to sacrifice on test day 5.

K. Clinical Observations

Daily animal health observations to detect moribund or dead mice and abnormal behavior and appearance among mice were conducted at least once daily throughout the study. Dead mice underwent a gross pathology examination. Careful clinical observations were performed prior to each dose (at approximately the same time ± 2 hours) and prior to sacrifice by individually handling and examining each animal for abnormal behavior and appearance.

L. Local Lymph Node Assay

Twenty-five µL of vehicle control, H-28072, or positive control were administered topically to the dorsum of each mouse ear for 3 consecutive days (test days 0-2) at dosages listed in the Study Design. Test days 3-4 were days of rest followed by intravenous injection of 20 µCi of ^{3}H-Thymidine per mouse on test day 5.

Approximately 5 hours after the injection, animals were sacrificed by carbon dioxide asphyxiation, draining auricular lymph nodes were removed, and single cell suspensions were prepared. The single cell suspensions were incubated at 2-8°C overnight. On test day 6, the single cell suspensions were counted on a beta counter and reported as disintegrations per minute (dpm).

A stimulation index (SI) was derived for each experimental group by dividing the mean dpm of each experimental group by the mean dpm of the vehicle control group. The decision process in regard to a positive response includes an SI of greater than or equal to 3.0 together with consideration of dose response and, where appropriate, statistical significance.

STATISTICAL ANALYSES

Significance was judged at $p < 0.05$ except for dpm data that were judged at $p < 0.01$. Lymph node dpm data were transformed to Log to obtain normality or homogenous variances.

		Method of Statistical Analysis	
Parameter	Preliminary Test	If preliminary test is not significant	If preliminary test is significant
Body Weight Body Weight Gain	Levene's test for homogeneity[6] and Shapiro-Wilk test[7] for normality[b]	One-way analysis of variance[8] followed by Dunnett's test[9,10,11]	Kruskal-Wallis test[12] followed by Dunn's test[13]

		Method of Statistical Analysis	
Parameter	Preliminary Test	If preliminary test is not significant	If preliminary test is significant
Lymph Node dpm Data[a]	Test for lack of trend[14]	Sequential application[15] of the Jonckheere-Terpstra trend test[16]	Preliminary tests for pairwise comparison
	OR[c]		
	Levene's test for homogeneity[6] and Shapiro-Wilk test[7] for normality[b]	One-way analysis of variance[8] followed by Dunnett's test[9,10,11]	Kruskal-Wallis test[12] followed by Dunn's test[13]

a Positive control data were not included in the statistical analysis of the test substance groups.
b If the Shapiro-Wilk test was not significant but Levene's test was significant, a robust version of Dunnett's test was used. If the Shapiro-Wilk test was significant, Kruskal-Wallis test was followed by Dunn's test.
c Pairwise comparisons and associated preliminary tests were only conducted if the test for lack of trend was significant.

When possible, an EC3 value for the SI data was derived from linear interpolation of points on the dose-response curve immediately above and below the 3-fold threshold. The equation used for calculation of EC3 was:

$$EC3 = c + [(3 - d)/(b - d)] \times (a - c)$$

where:

a = the lowest concentration giving stimulation greater than 3
b = the actual SI caused by a
c = the highest concentration failing to produce an SI of 3
d = the actual SI caused by c

RESULTS AND DISCUSSION

A. Body Weights, Body Weight Gains, and Clinical Signs of Toxicity

(Tables 1-3, Appendices B-C)

No statistically significant differences in mean body weights compared to the vehicle control group were observed at any test concentration. A statistically significant increase in mean body weight gains compared to the vehicle control group was observed at the 25% test concentration. No clinical signs of toxicity were observed in the study. One mouse in the 100% test concentration group was found dead on test day 3.

B. Stimulation Index Data

(Table 4, Appendix D)

Although statistically significant increases in cell proliferation measurements compared to the vehicle control group were observed at the 50% and 100% test concentrations, SIs of less than 3.0 were observed at all test concentrations of H-28072. Therefore, the EC3 value (the estimated concentration required to induce a threshold positive response, i.e., SI = 3) for the test substance under the conditions of this study was not calculable. A 25% concentration of the positive control, HCA, produced a dermal sensitization response in mice. Therefore, the LLNA test system was valid for this study with H-28072. Under the conditions of this study, H-28072 did not produce a dermal sensitization response in mice.

CONCLUSIONS

Based on these data, H-28072 is not a dermal sensitizer.

RECORDS AND SAMPLE STORAGE

Specimens (if applicable), raw data, the protocol, amendments (if any), and the final report will be retained at Haskell Laboratory, Newark, Delaware, or at Iron Mountain Records Management, Wilmington, Delaware.

REFERENCES

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TABLES

TABLES

EXPLANATORY NOTES

ABBREVIATIONS:

Mean Body Weights
Mean Body Weight Gains
Summary of Clinical Observations
Stimulation Index Data

dpm - disintegrations per minute
n - number of animals evaluated
N/A - not applicable
S.D. - standard deviation
SI - stimulation index

STATISTICAL ANALYSES:

Mean Body Weights
Mean Body Weight Gains

\# - Statistically significant difference from vehicle control at $p < 0.05$ by Jonckheere-Terpstra trend test.
* - Statistically significant difference from vehicle control at $p < 0.05$ by Dunnett/Tamhane-Dunnett test.
@ - Statistically significant difference from vehicle control at $p < 0.05$ by Dunn's test.
~ - Due to lack of vehicle control values or variability among group means, statistical analyses were unable to be performed.

Stimulation Index Data

\# - Statistically significant increase in dpm data from vehicle control at $p < 0.01$ by Jonckheere-Terpstra trend test.
* - Statistically significant increase in dpm data from vehicle control at $p < 0.01$ by Dunnett/Tamhane-Dunnett test.
@ - Statistically significant increase in dpm data from vehicle control at $p < 0.01$ by Dunn's test.

Table 1
Mean Body Weights of Female Mice

DAYS ON TEST	MEAN BODY WEIGHTS (g) Group 1 0%[a]	Group 2 5%	Group 3 25%	Group 4 50%	Group 5 100%	Group 6 25%[b]
0	21.7 1.4(5)	22.1 1.6(5)	22.0 1.6(5)	22.1 1.3(5)	21.7 1.4(5)	21.6 1.5(5)
5	22.0 0.9(5)	23.1 1.2(5)	24.0 1.3(5)	23.9 2.1(5)	21.4 1.4(4)	22.1 1.3(5)

Data arranged as: Mean
Standard deviation (Number of values included in calculation)

a Vehicle control
b Positive control

Table 2
Mean Body Weight Gains of Female Mice

DAYS ON TEST	MEAN BODY WEIGHT GAINS (g) Group 1 0%[a]	Group 2 5%	Group 3 25%	Group 4 50%	Group 5 100%	Group 6 25%[b]
0 - 5	0.4 1.2(5)	1.0 0.7(5)	2.0* 1.1(5)	1.8 1.1(5)	-0.5 0.4(4)	0.5 0.8(5)

Data arranged as: Mean
Standard deviation (Number of values included in calculation)

a Vehicle control
b Positive control

Table 3
Summary of Clinical Observations

Day numbers relative to Start Date

Sex: Female

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
	0%	5%	25%	50%	100%	25%
Animal Count	5	5	5	5	5	5

No Abnormality Detected

Table 4
Stimulation Index Data

GROUP	MATERIAL TESTED	n	MEAN (dpm)	S.D. (dpm)	SI
1	0% Vehicle Control	5	685.65	165.43	N/A
2	5%	5	653.85	248.19	0.95
3	25%	5	1064.25	386.08	1.55
4	50%	5	1599.05#	399.13	2.33
5	100%	4[b]	1569.50#	592.08	2.29
6	25% Positive Control[a]	5	6154.25	747.70	8.98

a Data were not included in the statistical analysis of the test substance groups.
b One mouse was found dead on test day 3.

APPENDICES

Appendix A
Certificate of Analysis

E. I. du Pont de Nemours and Company
Wilmington, DE 19898
USA

CERTIFICATE OF ANALYSIS

This Certificate of Analysis fulfills the requirement for characterization of a test substance prior to a study subject to GLP regulations. It documents the identity and content of the test substance. This work was conducted under EPA Good Laboratory Practice Standards (40 CFR 792).

Haskell Code Number	H-28072
Common Name	HFPO Dimer Acid Ammonium Salt
Purity Percent	82.6%
Other Components	Water – 13.9% Ammonium (excess) – 3.5%
Date of Analysis	July 19, 2007
Recommended reanalysis interval	1 year
Instructions for storage	NRT&H
Reference	DuPont-23285
Analysis performed at	E. I. DuPont de Nemours and Company DuPont Haskell Laboratories Newark, Delaware USA

Peter A. Bloxham, Ph.D.
Analyst's Name

Analyst's signature

23-JULY-2007
Date

Revision #1
July 20, 2007

Appendix B
Individual Body Weights

INDIVIDUAL BODY WEIGHTS

EXPLANATORY NOTES

ABBREVIATIONS:

g - grams
N - number of values included in calculation
S.D. - standard deviation

Individual Body Weights

Group Sex	Animal Number	Bodyweight (g) Day numbers relative to Start Date 0	5
1f	106	23.3	22.6
	107	20.7	22.5
	108	23.1	22.7
	109	20.9	20.5
	110	20.3	21.8
	Mean	21.66	22.02
	S.D.	1.42	0.92
	N	5	5
2f	206	20.6	22.4
	207	23.8	24.9
	208	23.1	23.2
	209	22.9	23.5
	210	20.1	21.7
	Mean	22.10	23.14
	S.D.	1.64	1.21
	N	5	5
3f	306	22.8	23.0
	307	20.8	24.1
	308	23.2	25.1
	309	19.9	22.4
	310	23.3	25.5
	Mean	22.00	24.02
	S.D.	1.55	1.33
	N	5	5

Individual Body Weights

Group Sex	Animal Number	Bodyweight (g) Day numbers relative to Start Date 0	5
4f	406	20.5	21.8
	407	20.9	22.0
	408	22.8	23.9
	409	23.5	25.2
	410	22.9	26.6
	Mean	22.12	23.90
	S.D.	1.33	2.06
	N	5	5
5f	506	21.1	20.3
	507	23.0	22.7
	508	20.1	20.0
	509	20.8	.
	510	23.4	22.6
	Mean	21.68	21.40
	S.D.	1.44	1.45
	N	5	4
6f	606	20.8	22.6
	607	23.0	23.2
	608	20.9	21.2
	609	20.0	20.3
	610	23.3	23.0
	Mean	21.60	22.06
	S.D.	1.46	1.26
	N	5	5

Appendix C
Individual Clinical Observations and Mortality Records

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS

EXPLANATORY NOTES

ABBREVIATIONS:

X - present

Individual Clinical Observations and Mortality Records

Group Sex	Animal Number	Clinical Sign	Site	Day numbers relative to Start Date 0	1	2	3	5
1f	106	No Abnormalities Detected		X	X	X	.	X
		Scheduled sacrifice		.	.	.	.	X
	107	No Abnormalities Detected		X	X	X	.	X
		Scheduled sacrifice		.	.	.	.	X
	108	No Abnormalities Detected		X	X	X	.	X
		Scheduled sacrifice		.	.	.	.	X
	109	No Abnormalities Detected		X	X	X	.	X
		Scheduled sacrifice		.	.	.	.	X
	110	No Abnormalities Detected		X	X	X	.	X
		Scheduled sacrifice		.	.	.	.	X
2f	206	No Abnormalities Detected		X	X	X	.	X
		Scheduled sacrifice		.	.	.	.	X
	207	No Abnormalities Detected		X	X	X	.	X
		Scheduled sacrifice		.	.	.	.	X
	208	No Abnormalities Detected		X	X	X	.	X
		Scheduled sacrifice		.	.	.	.	X
	209	No Abnormalities Detected		X	X	X	.	X
		Scheduled sacrifice		.	.	.	.	X
	210	No Abnormalities Detected		X	X	X	.	X
		Scheduled sacrifice		.	.	.	.	X
3f	306	No Abnormalities Detected		X	X	X	.	X
		Scheduled sacrifice		.	.	.	.	X
	307	No Abnormalities Detected		X	X	X	.	X
		Scheduled sacrifice		.	.	.	.	X
	308	No Abnormalities Detected		X	X	X	.	X
		Scheduled sacrifice		.	.	.	.	X
	309	No Abnormalities Detected		X	X	X	.	X
		Scheduled sacrifice		.	.	.	.	X
	310	No Abnormalities Detected		X	X	X	.	X
		Scheduled sacrifice		.	.	.	.	X

Individual Clinical Observations and Mortality Records

Group Sex	Animal Number	Clinical Sign	Site	Day numbers relative to Start Date 0	1	2	3	5
4f	406	No Abnormalities Detected		X	X	X	.	X
		Scheduled sacrifice		.	.	.	.	X
	407	No Abnormalities Detected		X	X	X	.	X
		Scheduled sacrifice		.	.	.	.	X
	408	No Abnormalities Detected		X	X	X	.	X
		Scheduled sacrifice		.	.	.	.	X
	409	No Abnormalities Detected		X	X	X	.	X
		Scheduled sacrifice		.	.	.	.	X
	410	No Abnormalities Detected		X	X	X	.	X
		Scheduled sacrifice		.	.	.	.	X
5f	506	No Abnormalities Detected		X	X	X	.	X
		Scheduled sacrifice		.	.	.	.	X
	507	No Abnormalities Detected		X	X	X	.	X
		Scheduled sacrifice		.	.	.	.	X
	508	No Abnormalities Detected		X	X	X	.	X
		Scheduled sacrifice		.	.	.	.	X
	509	No Abnormalities Detected		X	X	X	.	.
		Found dead		.	.	.	X	X
	510	No Abnormalities Detected		X	X	X	.	X
		Scheduled sacrifice		.	.	.	.	X
6f	606	No Abnormalities Detected		X	X	X	.	X
		Scheduled sacrifice		.	.	.	.	X
	607	No Abnormalities Detected		X	X	X	.	X
		Scheduled sacrifice		.	.	.	.	X
	608	No Abnormalities Detected		X	X	X	.	X
		Scheduled sacrifice		.	.	.	.	X
	609	No Abnormalities Detected		X	X	X	.	X
		Scheduled sacrifice		.	.	.	.	X
	610	No Abnormalities Detected		X	X	X	.	X
		Scheduled sacrifice		.	.	.	.	X

Appendix D
Individual Animal Cell Proliferation Data

INDIVIDUAL ANIMAL CELL PROLIFERATION DATA

EXPLANATORY NOTES

ABBREVIATIONS:

dpm - disintegrations per minute

FOOTNOTES:

a This mouse was found dead prior to this evaluation.

Individual Animal Cell Proliferation Data

Animal	dpm
Female, 1 - 0% Vehicle Control	
106	858.25
107	498.25
108	545.25
109	843.25
110	683.25
Female, 2 - 5% H-28072	
206	497.25
207	950.25
208	436.25
209	897.25
210	488.25
Female, 3 - 25% H-28072	
306	818.25
307	1447.25
308	1438.25
309	1052.25
310	565.25
Female, 4 - 50% H-28072	
406	1929.25
407	1665.25
408	1588.25
409	932.25
410	1880.25
Female, 5 - 100% H-28072	
506	2023.25
507	705.25
508	1689.25
509	[a]
510	1860.25
Female, 6 - 25% Positive Control	
606	7202.25
607	5844.25
608	6633.25
609	5356.25
610	5735.25